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ACC # 734916  
DP-MS-77-123

PLUTONIUM UPTAKE BY THE GREEN ALGA *Scenedesmus obliquus*  
(TÜRP) KÜTZ AS A FUNCTION OF ISOTOPE AND OXIDATION STATE

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A paper proposed for publication in  
*Environmental and Experimental Botany*

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This document was prepared in conjunction with work accomplished under Contract No.  
DE-AC09-76SR00001 with the U.S. Department of Energy.

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(TÜRP) KÜTZ AS A FUNCTION OF ISOTOPE AND OXIDATION STATE

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ABSTRACT

Uptake of  $^{238}\text{Pu}^{4+}$ ,  $^{238}\text{Pu}^{6+}$ ,  $^{239}\text{Pu}^{4+}$  and  $^{239}\text{Pu}^{6+}$  by the green alga *Scenedesmus obliquus* (Türp) Kütz was studied to determine whether isotope or oxidation state differences affect Pu uptake from aqueous medium by algal cells. At equivalent  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$  concentrations, even when oxidation states differed, accumulations of these isotopes by *S. obliquus* were not significantly ( $p > 0.05$ ) different. Plutonium accumulation by *S. obliquus* was log-linear. Liquid scintillation detectors coupled to pulse-height analyzers were suitable for determining the alpha activity of samples and were accurate over a wide range of sample activities. Plutonium-237 was an effective tracer for determining plutonium chemical recovery.

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## INTRODUCTION

Though the separations chemistry of plutonium has been studied extensively, little is known about the environmental chemistry<sup>(24)</sup> of this potentially hazardous material.<sup>(32)</sup> Knowledge and understanding of the complex cycling of plutonium in the environment is necessary to predict the effects of possible releases.<sup>(7)</sup> Plutonium can exist as 15 isotopes with atomic masses between 232 and 246. At the present time, the isotopes of environmental importance are  $^{238}\text{Pu}$ ,  $^{239}\text{Pu}$ , and  $^{240}\text{Pu}$ . Plutonium can exist simultaneously in four valence states in solution (3+, 4+, 5+, and 6+).<sup>(23)</sup>

Recent studies have raised the question of differential availability of  $^{237}\text{Pu}$ ,  $^{238}\text{Pu}$ , and  $^{239}\text{Pu}$  to biological systems and of differential mobility in soil.<sup>(14,17,22)</sup> The difference in availability of plutonium isotopes may be attributed to several possible mechanisms. Oxidation state has been shown to be important in determining plutonium availability to plants,<sup>(24)</sup> and Bondietti et al.<sup>(8)</sup> suggest that knowledge of plutonium oxidation state may help to explain environmental findings.

Kinetic isotope effects are generally small, and depend on the relative magnitude of mass difference between the isotopes.<sup>(18)</sup> Kinetic isotope effects between  $^{237}\text{Pu}$ ,  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$  are expected to be negligible. The major difference between the isotopes is in specific activity and decay energy. The specific activities (Ci/g) are  $^{237}\text{Pu}$ , 12,080;  $^{238}\text{Pu}$ , 17.2; and  $^{239}\text{Pu}$ , 0.058. Plutonium-237 decays predominantly by electron capture (99%), while  $^{238}\text{Pu}$  and

$^{239}\text{Pu}$  decay by alpha emission. The decay energies are 0.22 MeV for  $^{237}\text{Pu}$ , 5.59 MeV for  $^{238}\text{Pu}$ , and 5.24 MeV for  $^{239}\text{Pu}$ .<sup>(19)</sup> These differences in decay properties can cause differential availability of the isotopes by at least three mechanisms

- Recoil: The greater decay energy of  $^{238}\text{Pu}$  may result in increased ejection of atoms or clusters of atoms from  $\text{PuO}_2$  particles or of atoms sorbed onto substrates such as soil.<sup>(15)</sup> The ejected atoms may be more mobile in the environment.
- Oxidation: The higher specific activity and greater decay energy of  $^{238}\text{Pu}$  may provide opportunities for local oxidation to  $\text{Pu}^{6+}$  by radiolysis products.<sup>(9)</sup>  $\text{Pu}^{6+}$  is more available to plants<sup>(25)</sup> and is more mobile in soil.<sup>(26)</sup>
- Polymerization: The formation of polymeric hydrous oxides of plutonium is dependent on the plutonium concentration.<sup>(11,1)</sup> The plutonium concentration when  $^{237}\text{Pu}$  is used in experiments is usually much lower than it is when  $^{238}\text{Pu}$  or  $^{239}\text{Pu}$  is used. This may result in formation of plutonium polymer with  $^{238}\text{Pu}$  or  $^{239}\text{Pu}$  but not with  $^{237}\text{Pu}$ , resulting in increased availability of  $^{237}\text{Pu}$ .<sup>(6)</sup>

These mechanisms of differential availability of Pu isotopes will apply only when the isotopes are not homogeneously mixed. If the isotopes are present as a homogeneous mixture, the above mechanisms may result in increased plutonium availability but not in differential isotope availability.

This paper discusses an investigation of a simple interaction between a biological system and Pu. Specifically, an axenic algal culture, *Scenedesmus obliquus*, was exposed to the 4+ and 6+ oxidation states of  $^{238}\text{Pu}$  and  $^{239-240}\text{Pu}$  (hereafter referred to as  $^{239}\text{Pu}$ ) at three Pu concentration levels, to determine the effects of oxidation state and isotope. Phytoplankton is one of the first trophic levels in which soluble compounds are involved, and algal species can quickly accumulate various trace elements including radionuclides deposited in aquatic systems.<sup>(13)</sup> *Scenedesmus obliquus* is a commonly occurring species in many fresh water systems, and is, therefore, of ecological importance. Wahlgren and Nelson<sup>(30)</sup> reported that sorption of plutonium by phytoplankton may be responsible for the seasonal loss of plutonium from the epilimnion of Lake Michigan. Additional objectives of this study were to develop a rapid and inexpensive method of plutonium determination in aquatic systems and investigate the use of  $^{237}\text{Pu}$  as a tracer for chemical yield determinations during sample preparation and analysis.

## METHODS AND MATERIALS

### *Algal Uptake*

Axenic cultures of *Scenedesmus obliquus* (Türp) Kürtz (Strain No. 1592) were obtained from the Indiana University Culture Collection.<sup>(29)</sup> Cultures were checked for bacterial contamination by plating and microscopic examination. Stock cultures were grown in 4000-mL Erlenmeyer flasks containing 3500 mL of AAP culture

medium bubbled with sterile air.<sup>(2)</sup> Cultures were maintained at  $24 \pm 2^\circ\text{C}$  in a controlled-environment chamber with 4,035-lux illumination from balanced-spectrum Growlux fluorescent bulbs on a 12 h of light - 12 h of dark regime. Cells were harvested from early stationary phase by continuous-flow centrifugation and resuspended in 500 mL of fresh AAP medium. Cells were centrifuged at 12062 RCF for 10 minutes over 28% by weight bis-2(ethylhexyl) sebacate in N-butyl phthalate to separate viable cells from dead cells and cell fragments. The recovered cells were resuspended in 350 mL of AAP medium, resulting in a stock with a density of  $1 \times 10^7$  cells/mL.

Plutonium-238 and  $^{239}\text{Pu}$  solutions were obtained from the Analytical Chemistry Division of the Savannah River Laboratory. The  $^{237}\text{Pu}$  solution was obtained from Oak Ridge National Laboratory. The  $^{237}\text{Pu}$  contained 1.8 nCi/ $\mu\text{C}$   $^{237}\text{Pu}$  gross alpha activity. Results of analysis of the  $^{237}\text{Pu}$  are shown in Table 1. Plutonium solutions were prepared by dilution of stock  $^{238}\text{Pu}^{4+}$ ,  $^{238}\text{Pu}^{6+}$ ,  $^{239}\text{Pu}^{4+}$ , and  $^{239}\text{Pu}^{6+}$  solutions. The  $\text{Pu}^{4+}$  stock solutions were prepared by evaporating a Pu solution to dryness and dissolving the residue in 10 mL of 1N  $\text{HNO}_3$ . The solution was heated to  $70^\circ\text{C}$  in a water bath and 0.5 mL of 1M  $\text{NaNO}_2$  was added. The solution was maintained at  $70^\circ\text{C}$  for one hour and then diluted to 25 mL with 1N  $\text{HNO}_3$ .  $\text{Pu}^{6+}$  was prepared by adding 0.05 mL of 0.2M  $\text{KMnO}_4$  to 25 mL of a Pu solution (in 1N  $\text{HNO}_3$ ). The solution was allowed to stand for two hours and then 0.3M  $\text{MnCl}_2$  was added dropwise until

MnO<sub>2</sub> formed. The solution was allowed to stand for an additional two hours and was then filtered through a 0.45- $\mu$ m filter. The purities of the oxidation states were checked by extraction into thenoyl trifluoroacetone (TTA) from 1N HNO<sub>3</sub>. Under these conditions, only Pu<sup>4+</sup> will extract.<sup>(11)</sup> TTA-xylene extracted >97% of the plutonium from the Pu<sup>4+</sup> solutions and <0.1% from the Pu<sup>6+</sup> solutions.

Plutonium sorption by algal cells was studied in silicone-coated glass, 250-mL Erlenmeyer flasks containing 93 mL of sterile AAP medium. One mL of plutonium solution in 1N HNO<sub>3</sub> was pipetted into each flask and the pH was adjusted to 5.5 with 1N NaOH. Five mL of algal suspension were added, resulting in a total volume of 100 mL and an algal cell density of approximately  $5.5 \times 10^5$  cells/mL. The ionic strength (I) of the culture medium was 0.0143. Using the Davies approximation, the resulting activity coefficients for Pu<sup>4+</sup>, PuO<sub>2</sub><sup>2+</sup>, PuO<sub>2</sub>OH<sup>+</sup>, and PuO<sub>2</sub>(OH)<sub>3</sub><sup>-</sup> were 0.27, 0.72, 0.92, and 0.92, respectively.

After a 4-h incubation, two 5-mL aliquots were taken for microscopic cell density determination and plating loss. One 10-mL aliquot was removed from control flasks to determine plating loss in the absence of algal cells. The remaining algal suspension was transferred, in 30-mL aliquots, to a polyethylene centrifuge tube and centrifuged at 12062 RCF for 15 minutes over 28% by weight bis-2-(ethylhexyl) sebacate in N-butyl phthalate.<sup>(12)</sup> The supernate was aspirated off and retained. The algal cells and bis-2-(ethylhexyl) sebacate were washed from the centrifuge tube with



acetone and retained separately.

A  $^{239}\text{Pu}$  concentration comparable to the lowest  $^{238}\text{Pu}$  concentration is approximately 0.4 d/m/mL which is below detectability of the counting apparatus.  $^{239}\text{Pu}$ , therefore, did not overlap at this level. No attempt was made to achieve a  $^{238}\text{Pu}$  concentration similar to the highest  $^{239}\text{Pu}$  level, because that high activity would present counting problems and safety hazards. In addition, there was one control blank per treatment combination containing no algae. The experimental units were arranged in a  $2 \times 2 \times 3$  design with 5 replicates of each treatment combination (Table 2). Thus, the experiment consisted of 60 experimental units and 12 control blanks. However, because the centrifuge only held eight samples concurrently, samples were randomly blocked orthogonally into groups of eight and spaced 1-1/2 h apart for incubation and algal cell harvesting. Handling of controls was similar to that of experimentals, to evaluate possible contamination during the separation procedure.

#### *Analytical Procedures*

Radiochemical analysis involved a combination of wet and dry ashing (Boni, A. L. personal communication, 1976). The  $^{237}\text{Pu}$  stock solution (Table 1) was diluted 1000-fold, and one-mL aliquots were added to each sample before ashing. Samples were evaporated to dryness in 10 mL of 8N  $\text{HNO}_3$ . Following dry ashing at  $500^\circ\text{C}$  in a muffle furnace for six hours, samples were twice dissolved in 15 mL of 8N  $\text{HNO}_3$  and taken to dryness. The final residue was dissolved in 3 mL of hot 1N  $\text{HNO}_3$  and a 2-mL aliquot was pipetted into

a liquid scintillation vial containing 20 mL of Aquasol-2 scintillation solution (New England Nuclear). Samples were counted for 10 to 1000 minutes, depending upon sample activity.

Two liquid scintillation systems were used for plutonium measurement by alpha counting: 1) Nuclear Chicago liquid scintillation counter interfaced to an Ortec multichannel analyzer; and 2) Packard Tri-Carb liquid scintillation counter interfaced to a Technical Measurements Company multichannel analyzer. The liquid scintillation systems had backgrounds of 23 cpm with 2 SE minimum detectability of 0.5 cpm. Although these systems did not have the excellent pulse resolution characteristics of custom experimental systems,<sup>(20)</sup> they detected low-activity  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$  samples quite effectively.  $^{237}\text{Pu}$  tracer concentration in each sample was determined using a 20-cm NaI well-type detector. Taking the small  $^{237}\text{Pu}$  alpha contribution (Table 1) into account, the total sample plutonium was calculated using Equation 1.

$$\text{Total sample plutonium (d/m)} = \left[ \text{sa}^{\text{Pu}}_{\alpha} - \left( \frac{^{237}\text{Pu}_{\alpha}}{\text{st}^{237}\text{Pu}_{\gamma}} \right) \left( \frac{^{237}\text{Pu}_{\gamma}}{\text{st}^{\text{Pu}}_{\gamma}} \right) \right] \left( \frac{^{237}\text{Pu}_{\gamma}}{\text{sa}^{\text{Pu}}_{\gamma}} \right) \quad (1)$$

where:

$\text{sa}^{\text{Pu}}_{\alpha}$  and  $\text{st}^{237}\text{Pu}_{\alpha}$  = net alpha counts of sample and

$^{237}\text{Pu}$  standard in counts/min

$\text{sa}^{\text{Pu}}_{\gamma}$  and  $\text{st}^{237}\text{Pu}_{\gamma}$  = net gamma counts of the sample and

$^{237}\text{Pu}$  standard in counts/min

Data were converted from alpha decays/min per sample to plutonium atoms using Equation 2.

$$\text{Pu atoms} = \frac{d/m}{SW(2.22 \times 10^{12}) \text{ d/m/Ci}} \cdot A \quad (2)$$

where:

$d/m$  = alpha activity of sample (decays/min)

$S$  = specific activity of isotope (Ci/g)

$W$  = atomic mass of isotope (g/gram atom)

$A$  = Avogadro's number ( $6.023 \times 10^{23}$  atoms/gram atom)

Plutonium in solution is reported as plutonium atoms/mL and plutonium associated with algal cells is reported as plutonium atoms/cell which facilitates direct comparison of  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$ . Thus, the "pCi/g" term was eliminated as it presented a distorted representation of isotopic behavior when comparing isotopes of widely differing half-lives, such as  $^{238}\text{Pu}$  (88 yr) and  $^{239}\text{Pu}$  (24,000 yr).

## RESULTS

The experimental results of plutonium uptake by the green alga *S. obliquus* are summarized in Figure 1. The amount of  $^{238}\text{Pu}^{4+}$  and  $^{238}\text{Pu}^{6+}$  associated with algal cells was log-linearly proportional to Pu concentration in the medium. No significant ( $p > 0.05$ ) oxidation state effect on algal sorption of  $^{238}\text{Pu}$  or  $^{239}\text{Pu}$  was observed at the three concentrations studied. Likewise, no significant ( $p > 0.05$ ) difference between the algal uptake of  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$  was observed at similar concentrations. Approximately 20 percent of the plutonium was observed associated with the algal fraction.

Plutonium is also known to "plate out" on vessel walls and be

effectively removed from solution. However, when each entire solution and its associated algal fraction were assayed and taken together, a 96.5% mass balance was obtained, indicating a rather small loss due to adsorption on vessel walls.

## DISCUSSION

The experimental plutonium concentrations are relevant to levels encountered in the environment. The lowest  $^{238}\text{Pu}$  level studied ( $10^8$  atoms/mL) is just above plutonium concentrations reported in the Savannah River (approximately  $10^7$  atoms/mL).<sup>(3)</sup> The maximum permissible  $^{239}\text{Pu}$  concentration in drinking water<sup>(16)</sup> is  $10^9$  atoms/mL.

Plutonium can coexist in solution at all four oxidation states<sup>(4)</sup> between  $\text{Pu}^{4+}$  and  $\text{Pu}^{6+}$ . The distribution of oxidation states at equilibrium is dependent on the pH and Eh of the solution and on the degree of complexation.<sup>(28)</sup> The estimated oxidation state distribution in the AAP medium (pH = 5.5, Eh = 0.35v, 0.01M  $\text{NO}_3^-$ ) is 100%  $\text{Pu}^{3+}$ . However, the rate at which equilibrium is attained will be different depending on the initial oxidation state. No analyses were conducted subsequent to the experiments to verify that the oxidation state of the isotopes had remained constant throughout the 4-h experiment. Quadrivalent plutonium will be reduced to  $\text{Pu}^{3+}$  more readily than will  $(\text{PuO}_2)^{2+}$  because two plutonium-oxygen bonds must be broken in the latter case.<sup>(11)</sup> Therefore, it is unlikely that both  $\text{Pu}^{4+}$  and  $(\text{PuO}_2)^{2+}$  converted in exactly the same manner during the four-hour incubation.

Alberts (personal communication, 1977\*) found  $\text{Pu}^{6+}$  to be the most stable oxidation state in filtered and synthetic Lake Michigan water.

Plutonium ions will form complexes with nitrate ions in solution. The degree of complexation is dependent on the oxidation state of the Pu and  $\text{NO}_3^-$  concentration.<sup>(21)</sup> The order of stability of nitrate complexes<sup>(11)</sup> is  $\text{Pu}^{4+} > \text{Pu}^{3+} > (\text{PuO}_2)^{2+} > (\text{PuO}_2)^+$ . While  $\text{Pu}^{4+}$  readily forms nitrate complexes, nitrate exhibits only a slight tendency<sup>(11)</sup> to form complexes with  $\text{Pu}^{6+}$ . The degree of complexation in the AAP medium is difficult to estimate because of the low ionic strength and low  $\text{NO}_3^-$  concentration, since reported Pu stability constants were measured in solutions of ionic strength 1M or greater and at relatively high  $\text{NO}_3^-$  concentrations.<sup>(11)</sup> If, due to kinetic considerations, equilibrium had not been established during the 4-h incubation, the solubility, extent of hydrolysis and degree of nitrate complexation of plutonium in the two solutions (initially  $\text{Pu}^{4+}$  and  $\text{Pu}^{6+}$ ) could be considerably different.<sup>(27)</sup>

Plutonium is known to polymerize in weakly acidic solutions resulting in polymers<sup>(11)</sup> of  $\text{Pu}^{4+}$  which can be removed by centrifugation at 3600 rpm for 2.5 h. However, polymerization is dependent upon Pu concentration as well as pH. If only small quantities of Pu are available, a high pH is required to induce polymerization. Thus, polymerization was probably not an important factor at the Pu concentrations studied here.

The results of this study show that from a practical standpoint of predicting environmental behavior in a water-phytoplankton

system, whether the  $\text{Pu}^{4+}$  and  $\text{Pu}^{6+}$  solutions reached equilibrium (resulting in the same distribution of oxidation states) or not is immaterial. In either case, whether uptake is independent of oxidation state or equilibrium is reached in as little as four hours, the result, Pu absorption by algae, would be the same. The distribution coefficients of  $\text{Pu}^{6+}$  and  $\text{Pu}^{4+}$  in seston and sediments are such that they suggest that  $\text{Pu}^{6+}$  may be reduced to  $\text{Pu}^{4+}$  by algal cells at the cell wall (Alberts, personal communication, 1977). Such a mechanism could also explain the results observed here. For determining and modeling plutonium uptake by algae, a knowledge of the  $(\text{CO}_3^{2-})$  is necessary for determining plutonium availability to phytoplankton.<sup>(10)</sup>

Bair et al.<sup>(6)</sup> found differential mobility of  $^{237}\text{Pu}$  and  $^{239}\text{Pu}$  in dogs. Bair<sup>(5)</sup> also found  $^{238}\text{PuO}_2$  translocation in dogs to be greater than  $^{239}\text{PuO}_2$ . While this biological system is much different than that studied here, it does indicate differential behavior due to mass differences. However, Wayman and Bartelt<sup>(31)</sup> reported no significant difference in  $^{238}\text{Pu}$  and  $^{239}\text{Pu}$  concentration factors in fish, when expressed on a gram atomic mass basis. Their results agree closely with those of this study, which indicates no effect of Pu mass on sorption by algal cells.

The sample preparation and counting methods were simple, inexpensive, and suitable for this experiment.  $^{237}\text{Pu}$ , as the internal spike, eliminated the need for sophisticated counting systems, such as high-resolution alpha spectroscopy systems that are required when  $^{236}\text{Pu}$  or  $^{242}\text{Pu}$  are used as the internal spike. The

technique has applicability to a variety of aquatic experimental designs where the gross alpha content is solely from plutonium added by the experimenter.

#### ACKNOWLEDGEMENTS

We are indebted to Ms. A. Gibbs for help with liquid scintillation analyses, and Dr. R. M. Wallace for the use of his isotope laboratory. Mr. N. D. Johnson helped with  $^{237}\text{Pu}$  analyses and Ms. L. A. Briesse helped with algal cultures. The manuscript was critically reviewed by J. J. Alberts, K. W. McLeod, M. H. Smith, and W. B. Vernberg.

Work was supported by Contracts AT(07-2)-1 and EY-76-C-09-0819 with the U.S. Department of Energy.

## REFERENCES

1. Andelman, J. B. and Rozzell, T. C. (1970). Plutonium in the water environment. In: *Radionuclides in the Environment*, E. C. Freiling (ed.); American Chemical Society, Washington, D. C.
2. Anon. (1971). Algal assay procedure bottle test. *National Eutrophication Research Program*. Environmental Protection Agency. 82 pp.
3. Anon. (1976). *Environmental Monitoring in the Vicinity of the Savannah River Plant*. USERDA Report DPSPU-77-30-1, E. I. du Pont de Nemours & Company, Savannah River Laboratory, Aiken, S. C.
4. Bagnall, K. W. (1972). The actinide elements. In: *Topics in Inorganic and General Chemistry*. P. L. Robinson (ed.), monograph 15, Elsevier Publishing Company, Amsterdam.
5. Bair, W. J., Ballou, J. E., Park, J. F., and Sanders, C. L. (1973). Plutonium in soft tissues with emphasis on the respiratory tract. In: *Uranium Plutonium Transplutonic Elements*, Hodge, H. C., Stannard, J. W. and Hursh, J. B (eds.). Springer-Verlag, New York. 995 p.
6. Bair, W. J., Williard, D. H., Nelson, I. C., and Case, A. C. (1974). Comparative distribution and excretion of  $^{237}\text{Pu}$  and  $^{239}\text{Pu}$  nitrates in beagle dogs. *Health Physics* 27, 392-396.
7. Bennett, B. C. (1974). Environmental pathways of transuranium elements. In: *Plutonium and Other Transuranics: Sources,*



*Environmental Distribution and Biological Effects*, B. W.

Wachholtz (ed.) USAEC Report WASH-1359.

8. Bondietti, E. A., Reynolds, S. A., and Shanks, M. H. (1976).  
Interaction of plutonium with complexing substances in soils  
and natural waters, pp 273-287. In: *Proceedings of IAEA  
Symposium on Transuranium Nuclides in the Environment, November  
17-21, 1975, San Francisco, California*. Report IAEA-SM-199/51,  
International Atomic Energy Agency, Vienna.
9. Bondietti, E. A. and Reynolds, S. A. (1976). Field and labora-  
tory observations on plutonium oxidation states. In: *Proceed-  
ings of an Actinide-Sediment Reactions Working Meeting, February  
10-11, 1976*. USERDA Report BNWL-2117, Battelle-Northwest Lab-  
oratories, Richland, Washington.
10. Conway, H. L., Wahlgren, M. A., Peterson, N., and Nelson, D. M.  
(1976). The sorption of  $^{237}\text{Pu}$  by the diatom *Asterionella*  
*formosa*. *Radiological and Environmental Research Division Annual  
Report - Ecology*, pp 61-64. Argonne National Laboratory,  
Argonne, Illinois.
11. Cleveland, J. M. (1970). *The Chemistry of Plutonium*. Gordon  
and Breach Sci., Publ., New York.
12. Giesy, J. P. and Paine, D. (1977). Effects of naturally occur-  
ring aquatic fractions on  $^{241}\text{Am}$  uptake by *Scenedesmus obliquus*  
(Chlorophyceae) and *Aeromonas hydrophila* (Pseudomonadaceae),  
*Appl. Environ. Microbiol.* 33, 89-96.

13. Gromov, V. V. and Spitzyn, V. I. (1974). Uptake of plutonium, ruthenium, and technetium by phytoplankton. USAEC Report ORNL-tr 2907, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
14. Hakonson, T. E. and Johnson, L. J. (1973). *Distribution of Environmental Plutonium in the Trinity Site Ecosystem after 27 Years*. USAEC Report LA-UR-73-1291, Los Alamos Scientific Laboratory, NM.
15. Hanson, W. C. (1975). Ecological considerations of the behavior of plutonium in the environment. *Health Phys.* 28, 529.
16. *Environmental Protection Agency National Interim Primary Drinking Water Regulations*. 40 CFR 141; 40 FR59565, December 24, 1975; Amended by 41 FR 28402, July 9, 1976.
17. Hersloff, L. W. (1977). *Uptake of Three Plutonium Isotopes in a Plant-Soil System*. Ph.D. thesis, Colorado State University, Ft. Collins, Colorado.
18. Laidler, K. J. (1965). *Chemical Kinetics*. McGraw-Hill, N.Y.
19. Lederer, C. M. , Hollander, J. M. and Perlman, I. (1967). *Table of Isotopes, 6th ed.*, Wiley and Sons, Inc., New York.
20. McKlveen, J. W. and McDowell, W. J. (1975). Some studies of reflector construction and electronics configurations for optimizing pulse-height and pulse-shape resolution in alpha liquid-scintillation spectrometry. *Nucl. Technol.* 28, 159-164.

21. McLane, C. K., Dixon, J. S., and Hindman, J. C. (1949). Complex ions of plutonium. Transference measurements. In: *The Transuranium Elements*, Seaborg, G. T., Katz, J. J., and Manning, W. M. (eds.), National Nuclear Energy Series, IV., 14-B, pp. 358-369. McGraw-Hill Book Co., New York.
22. McLendon, H. R., Stewart, O. M., Boni, A. L., Corey, J. C., McLeod, K. W., and Pinder, J. E. (1975). Relationships among plutonium contents of soil, vegetation, and animals collected on and adjacent to an integrated nuclear complex in the humid southeastern United States of America. pp 347-363. In: *Proceedings of International Atomic Energy Agency Symposium on Transuranium Nuclides in the Environment, November 17-21, 1975, San Francisco, California*. Report IAEA-SM-199/85, International Atomic Energy Agency, Vienna.
23. Murray, C. N. and Fukai, R. (1975). Adsorption-desorption characteristics of plutonium and americium with sediment particles in the estuarine environment. pp 179-192. In: *Proceedings of an International Symposium on Radiological Impacts of Releases from Nuclear Facilities into Aquatic Environments*. Report IAEA-SM-198/25, International Atomic Energy Agency, Vienna.
24. Paine, D. (1974). *Plutonium in Aquatic Systems*. Ph.D. Thesis, Colorado State University, Fort Collins, Colorado. 176 pp.

25. Price, K. R. (1973). A review of transuranic elements in soils, plants, and animals. *J. Environ. Quality*, Vol. 2, No. 1, 1973.
26. Prout, W. E. (1958). Adsorption of radioactive wastes by Savannah River Plant soil. *Soil Science* 86, 13-17.
27. Rai, D. and Serne, R. J. (1977). Plutonium activities in soil solutions and the stability and formation of selected plutonium minerals. *J. Environ. Qual.* 6, 89-95.
28. Silver, G. L. (1971). Plutonium in natural waters. USAEC Report MLM 1870, Mound Laboratory, Miamisburg, OH.
29. Starr, R. C. (1971). Culture collection of algae at Indiana University - additions to the collection. July 1966, July 1977. *J. Phycol.* 7, 350-362.
30. Wahlgren, M. A. and Nelson, D. M. (1974). Studies of plutonium cycling and sedimentation in Lake Michigan. *Proc. 17th Conf. Great Lakes Res. Int. Assoc. Great Lakes Res.*, Ann Arbor, Michigan, pp 212-218.
31. Wayman, C. W. and Bartelt, G. E. (1977). Distribution of  $^{238}\text{Pu}$  in tissues of fish from the canal in Miamisburg, Ohio. (To be published.)
32. Zolotar, B. A. (1975). Plutonium recycle. In: *Energy and the Environment; Cost-Benefit Analysis*. R. A. Karam and K. Z. Morgan (eds.), Supplement I to Energy.

Table 1. Analysis of Plutonium-237 Product as of November 4, 1976

$^{237}\text{Pu}$	1.4 $\mu\text{Ci/ml}$
Solution	0.5 M HCl
$^{67}\text{Ga}$ (78 hr)	0.8 nCi/ $\mu\text{Ci}$ $^{237}\text{Pu}$
Gross Alpha Activity	1.8 nCi/ $\mu\text{Ci}$ $^{237}\text{Pu}$
Alpha-Spectrum Components	4.9 MeV $\sim 9.2\%$
	5.3 MeV $\sim 13.7\%$
	5.7 MeV $\sim 71.1\%$

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Table 2. Plutonium Nitrate Concentrations in Experimental Flasks<sup>a</sup>

Isotope and Oxidation State	Pu Concentration, atoms/mL
$^{238}\text{Pu}^{4+}$	$3.25 \times 10^8$ (n=5); $9.15 \times 10^9$ (n=5); $2.71 \times 10^{10}$ (n=5)
$^{238}\text{Pu}^{6+}$	$6.87 \times 10^8$ (n=5); $1.11 \times 10^{10}$ (n=5); $3.78 \times 10^{10}$ (n=5)
$^{239}\text{Pu}^{4+}$	$1.11 \times 10^{10}$ (n=4); $3.87 \times 10^{10}$ (n=5); $3.78 \times 10^{11}$ (n=3)
$^{239}\text{Pu}^{6+}$	$1.04 \times 10^{10}$ (n=3); $4.61 \times 10^{10}$ (n=5); $3.81 \times 10^{11}$ (n=5)

a. n = number of replicate flasks.

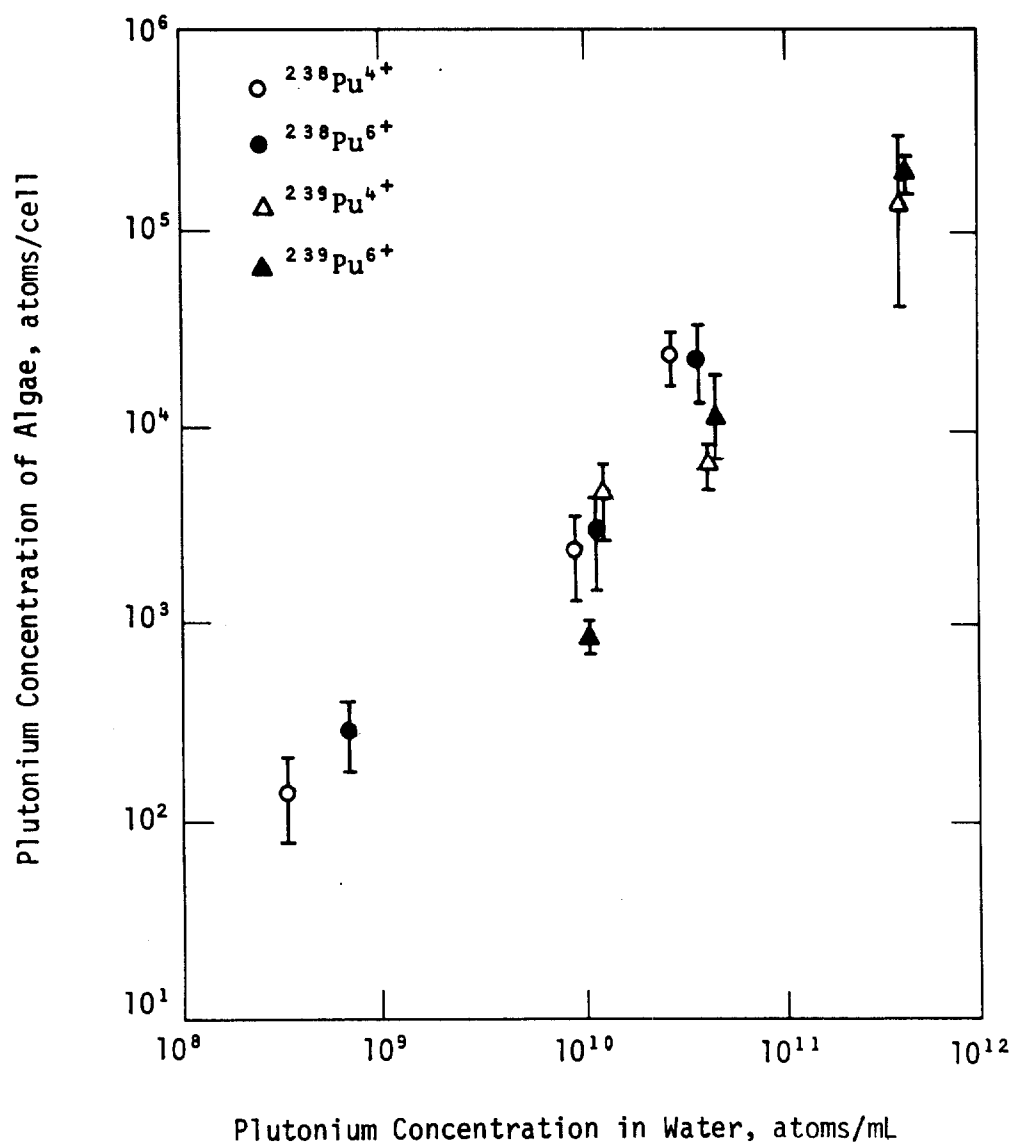


FIGURE 1. Plutonium Uptake by Green Alga *S. obliquus*